Contribution ID: **41** Type: **not specified**

Combined X-ray and neutron crystallography for drug design purposes

COVID-19, caused by SARS-CoV-2, remains a global health threat even with available vaccines and therapeutic options. The viral main protease (Mpro) is indispensable for the virus replication and thus is an important target for small-molecule antivirals. Computer-assisted and structure-based drug design strategies rely on atomic scale understanding of the target biomacromolecule traditionally derived from X-ray crystallographic data collected at cryogenic temperatures. Conventional protein X-ray crystallography is limited by possible cryo-artifacts and its inability to locate the functional hydrogen atoms crucial for understanding chemistry occurring in enzyme active sites. Neutrons are ideal probes to observe the protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges – crucial information for drug design. Our room-temperature X-ray crystal structures of Mpro provided insights into the reactivity of the catalytic cysteine, malleability of the active site, and binding modes with clinical protease inhibitors. The neutron crystal structures of ligand-free and inhibitor-bound Mpro were determined allowing the direct observation of protonation states of all residues in a coronavirus protein for the first time. This information was used to design nanomolar hybrid reversible covalent inhibitors with robust antiviral properties. High-throughput virtual screening, utilizing ORNL's supercomputing capabilities, in conjunction with in vitro assays identified a lead noncovalent compound with sub-micromolar affinity. The neutron structure of Mpro in complex with the noncovalent inhibitor was used in a structure-activity relationship (SAR) study guided by virtual reality structure analysis to novel Mpro inhibitors with improved affinity to the enzyme. A series of X-ray structures, and biophysical and biochemical measurements on specifically designed Mpro constructs that mimic the immature monomeric state of the enzyme provide insights into the mechanism of Mpro autoprocessing, a crucial step in the SARS-CoV-2 replication cycle and a novel target for drug design. Our research is providing real-time data for atomistic design and discovery of Mpro inhibitors to combat the COVID-19 pandemic and prepare for future threats from pathogenic coronaviruses.

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